

FORM PTO-1390 (REV. 10-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 20397P
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) <div style="font-size: 1.5em; font-weight: bold; text-align: center;">09/914106</div>
INTERNATIONAL APPLICATION NO. PCT/US00/04416	INTERNATIONAL FILING DATE February 22, 2000	PRIORITY DATE CLAIMED February 24, 1999
TITLE OF INVENTION G PROTEIN-COUPLED RECEPTOR RESEMBLING GALANIN RECEPTORS		
APPLICANT(S) FOR DO/EO/US Andrew D. Howard, Gary P. O'Neill, Brian O'Dowd and Susan George		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made and the US was elected by the expiration of the 19th month from the earliest claimed priority date (PCT Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed [35 U.S.C. 371(c)(2)]. <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed [35 U.S.C. 371(c)(2)]. 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]. <ol style="list-style-type: none"> a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)]. 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)]. 10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)]. <p>Items 11 to 16 below concern other document(s) or information included:</p> <ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input checked="" type="checkbox"/> Other items or information: 		

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U.S. APPLICATION NO. (If known, see 37 CFR 1.5) <div style="font-size: 1.5em; font-weight: bold; margin-top: 5px;">09/914106</div>		INTERNATIONAL APPLICATION NO PCT/US00/04416		ATTORNEY'S DOCKET NUMBER 20397P	
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17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE [37 CFR 1.492(a)(1)-(5)]: Neither international preliminary examination fee (37 CFR 1.482) nor international search fee [37 CFR 1.445(a)(2)] paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$1,000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.. . . . \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee [37 CFR 1.445(a)(2)] paid to USPTO \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... \$690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... \$100.00 <div style="text-align: right; margin-top: 5px;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>	CALCULATIONS	PTO USE ONLY																				
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Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(e)].	\$0.00																					
<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <th style="width:20%;">Claims</th> <th style="width:20%;">Number Filed</th> <th style="width:20%;">Number Extra</th> <th style="width:20%;">Rate</th> </tr> <tr> <td>Total Claims</td> <td>13 - 20 =</td> <td>0</td> <td>X \$18.00</td> </tr> <tr> <td>Independent Claims</td> <td>6 - 3 =</td> <td>3</td> <td>X \$80.00</td> </tr> <tr> <td>Multiple dependent claim(s) (if applicable)</td> <td></td> <td></td> <td>+ \$270.00</td> </tr> <tr> <td colspan="3" style="text-align: right;">TOTAL OF ABOVE CALCULATIONS =</td> <td></td> </tr> </table>	Claims	Number Filed	Number Extra	Rate	Total Claims	13 - 20 =	0	X \$18.00	Independent Claims	6 - 3 =	3	X \$80.00	Multiple dependent claim(s) (if applicable)			+ \$270.00	TOTAL OF ABOVE CALCULATIONS =				\$340.00	
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<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.																						
SUBTOTAL =	\$340.00																					
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(f)].	\$0.00																					
TOTAL NATIONAL FEE =	\$340.00																					
Fee for recording the enclosed assignment [37 CFR 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property.	\$0.00																					
TOTAL FEES ENCLOSED =	\$340.00																					
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a. ☐ A check in the amount of \$ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 13-2755 in the amount of \$340.00 to cover the above fees.
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**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive
 [37 CFR 1.137(a) or (b)] must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

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DATE: August 23, 2001

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SIGNATURE
 Jack L. Tribble
 NAME
32,633
 REGISTRATION NUMBER

TITLE OF THE INVENTION

G PROTEIN-COUPLED RECEPTOR RESEMBLING GALANIN RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

10 REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

15 This invention relates to novel human and rat DNAs encoding GPR54, a G protein-coupled receptor (GPCR) related to the galanin receptors, the proteins encoded by the DNAs, and methods of identifying selective agonists and antagonists of the proteins encoded by the DNAs.

BACKGROUND OF THE INVENTION

20 G-protein coupled receptors (GPCRs) are a very large class of membrane receptors that relay information from the exterior to the interior of cells. GPCRs function by interacting with a class of heterotrimeric proteins known as G-proteins. Most GPCRs function by a similar mechanism. Upon the binding of agonist, a GPCR catalyzes the dissociation of guanosine diphosphate (GDP) from the
25 α subunit of G proteins. This allows for the binding of guanosine triphosphate (GTP) to the α subunit, resulting in the disassociation of the α subunit from the β and γ subunits. The freed α subunit then interacts with other cellular components, and in the process passes on the extracellular signal represented by the presence of the agonist. Occasionally, it is the freed β and γ subunits which transduce the agonist
30 signal.

GPCRs possess common structural characteristics. They have seven hydrophobic domains, each about 20-30 amino acids long, linked by sequences of hydrophilic amino acids of varied length. These seven hydrophobic domains intercalate into the plasma membrane, giving rise to a protein with seven
35 transmembrane domains, an extracellular amino terminus, and an intracellular

carboxy terminus (Strader et al., 1994, Ann. Rev. Biochem. 63:101-132; Schertler et al., 1993, Nature 362:770-772l; Dohlman et al., 1991, Ann. Rev. Biochem. 60:653-688).

GPCRs are expressed in a wide variety of tissue types and respond to a wide range of ligands, *e.g.*, protein hormones, biogenic amines, peptides, lipid derived messengers, *etc.* Given their wide range of expression and ligands, it is not surprising that GPCRs are involved in many pathological states. This has led to great interest in developing modulators of GPCR activity that can be used pharmacologically. For example, Table 1 of Stadel et al., 1997, Trends Pharmacol. Sci. 18:430-437, lists 37 different marketed drugs that act upon GPCRs. Accordingly, there is a great need to understand GPCR function and to develop agents that can be used to modulate GPCR activity.

Galanin is widely distributed in the central and peripheral nervous system. Galanin in most species is a 29 amino acid peptide with an amidated carboxyl terminus. Human galanin is unique in that it is longer, 30 amino acids, and is not amidated. There is strong conservation of the galanin sequence, with the amino terminal fifteen residues being absolutely conserved in all species. Galanin immunoreactivity and binding is abundant in the hypothalamus, the locus coeruleus, the hippocampus, and the anterior pituitary, as well as regions of the spinal cord, the pancreas, and the gastrointestinal tract.

Injection of galanin into the paraventricular nucleus (PVN) of the hypothalamus produces a dose-dependent increase in feeding in satiated rats. Although galanin can enhance carbohydrate ingestion, studies have shown that it profoundly increases fat intake. It has been suggested that galanin shifts macronutrient preference from carbohydrate to fat. The same injections of galanin that increase feeding reduce energy expenditure and inhibit insulin secretion. There is enhanced galanin expression in the hypothalamus of genetically obese rats compared with their lean littermates. Injection of peptide galanin receptor antagonists into the PVN blocks the galanin-specific induction of increased fat intake. Specific galanin antisense oligonucleotides when injected into the PVN produce a specific decrease in galanin expression associated with a decrease in fat ingestion and total caloric intake while hardly affecting either protein or carbohydrate intake. Thus galanin appears to be a potential neurochemical marker related to the behavior of fat ingestion and galanin receptors are attractive targets for the development of drugs to treat obesity and other eating disorders.

Galanin inhibits cholinergic function and impairs working memory in rats. Lesions that destroy cholinergic neurons result in deficits in spatial learning tasks. While locally administered acetylcholine (ACh) reverses some of this deficit, galanin blocks this ACh-mediated improvement. Evidence from autopsy samples
5 from Alzheimer's disease-afflicted brains suggests an increased galinergic innervation of the nucleus basalis. Thus, if galinergic overactivity contributes to the decline in cognitive performance in Alzheimer's disease, galanin antagonists may be therapeutically useful in alleviating cognitive impairment.

Other physiological processes in which galanin has been implicated
10 include nociception (Verge et al., 1993, *Neurosci. Lett.* 149:193-197) and sexual behavior (Benelli et al., 1994, *Eur. J. Pharmacol.* 260:279-282).

In the rat, administration of galanin intracerebroventricularly, subcutaneously, or intravenously increases plasma growth hormone. Infusion of human galanin into healthy subjects also increases plasma growth hormone and
15 potentially enhances the growth hormone response to growth hormone releasing hormone (GHRH).

Galanin levels are particularly high in dorsal root ganglia. Sciatic nerve resection dramatically up-regulates galanin peptide and mRNA levels. Chronic administration of galanin receptor antagonists (M35, M15) after axotomy results in a
20 marked increase in self mutilation behavior in rats, generally considered to be a response to pain. Application of antisense oligonucleotides specific for galanin to the proximal end of a transected sciatic nerve suppressed the increase in galanin peptide levels with a parallel increase in autotomy. Galanin injected intrathecally acts synergistically with morphine to produce analgesia, this antinociceptive effect of
25 morphine is blocked by galanin receptor antagonists. Thus, galanin agonists may have some utility in relieving neural pain.

The actions of galanin are mediated by at least three high affinity galanin receptors that are coupled by pertussis toxin sensitive G_i/G_o proteins to inhibition of adenylate cyclase activity, closure of L-type Ca^{++} channels, and opening
30 of ATP-sensitive K^+ channels (Habert-Ortoli et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:9780-9783; Howard et al., 1997, *FEBS Lett.* 405:285-290; Wang et al., 1997, *J. Biol. Chem.* 272:31949-31952; Kolakowski et al., 1998, *J. Neurochem* 71:2239-2251). Specific binding of ^{125}I -galanin (K_d approximately 1 nM) has been demonstrated in areas paralleling localization of galanin immunoreactivity:
35 hypothalamus, ventral hippocampus, basal forebrain, spinal cord, pancreas, and

pituitary. In most tissues, the amino terminus (GAL 1-15) is sufficient for high affinity receptor binding and agonist activity.

A galanin receptor cDNA was isolated by expression cloning from a human Bowes melanoma cell line. (Habert-Ortoli, et al. 1994. Proc. Nat. Acad. Sci., USA 91: 9780-9783). This receptor, GALR1, is expressed in human fetal brain and small intestine, but little else is known of its distribution. Gal(1-16) is at least 1,000 times more active than pGAL(3-29) as an inhibitor of ¹²⁵I-porcine galanin binding to this receptor transiently expressed in COS cells. It remains to be determined whether this receptor subtype represents the hypothalamic receptor that mediates galanin specific feeding behavior.

Galanin receptors have been described in several international patent publications (WO 98/03548; WO 97/46681; WO 97/26853; WO 98/29439; WO 98/29440; WO 98/29441; WO 95/22608). European Patent Application EP 711830 also describes a galanin receptor.

It would be desirable to identify additional galanin receptors so that they can be used to further characterize this biological system and to identify galanin receptor subtype selective agonists and antagonists.

SUMMARY OF THE INVENTION

The present invention is directed to novel human and rat DNAs that encode a G-protein coupled receptor, GPR54. The DNAs encoding GPR54 are substantially free from other nucleic acids and have the nucleotide sequences shown as SEQ.ID.NO.:1 (human GPR54) and SEQ.ID.NO.:2 (rat GPR54). Also provided are GPR54 proteins encoded by the novel DNA sequences. The GPR54 proteins are substantially free from other proteins and have the amino acid sequences shown as SEQ.ID.NO.:3 (human GPR54) and SEQ.ID.NO.:4 (rat GPR54). Methods of expressing GPR54 in recombinant systems and of identifying agonists and antagonists of GPR54 are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-B shows the complete cDNA sequence and amino acid sequence of human GPR54. The DNA sequence shown is SEQ.ID.NO.:1. The amino acid sequence shown is SEQ.ID.NO.:3.

Figure 2A-B shows the complete cDNA sequence of rat GPR54 (SEQ.ID.NO.:2).

Figure 3 shows the complete amino acid sequence of human GPR54 (SEQ.ID.NO.:3).

Figure 4 shows the complete amino acid sequence of rat GPR54 (SEQ.ID.NO.:4).

5 Figure 5A-B shows the location of the rat GPR54 open reading frame. The nucleotide sequence shown is (SEQ.ID.NO.:2). The amino acid sequence shown is (SEQ.ID.NO.:4).

Figure 6 shows the results of a Northern blot of rat GPR54 mRNA in rat brain. Each lane contained 5 μ g of poly(A)⁺ RNA isolated from various tissues.

10 Figure 7A-D shows darkfield autoradiograms of sagittal and coronal sections of rat brain showing the localization of GPR54 receptor mRNA. Figure 7A shows a lateral representative section at 0.9 mm. Also shown are representative sections at levels relative to the bregma at -3.3 mm (Figure 7B), -3.8 mm (Figure 7C), and -6.3 mm (Figure 7D). Aco = cortical nucleus of the amygdala; Ahy = anterior
15 hypothalamic area; Arc = hypothalamic arcuate nucleus; IC = inferior colliculus; CA, field of Ammon's horn; DG, dentate gyrus; DM, dorsomedial hypothalamic nucleus; LC, locus coeruleus; LH, lateral hypothalamic area, LHb, lateral habenular nucleus; MeA, medial nucleus of the amygdala; MPO, medial preoptic area; MRN, mesencephalic reticular nucleus; PAG, periaqueductal gray; PB, parabrachial nucleus;
20 PF, parafascicular thalamic nucleus; PH, posterior hypothalamic nucleus; PMV, ventral premammillary nucleus; PO, primary olfactory cortex; RSpl, retrosplenial cortex; SC, superior colliculus; SHy, septohypothalamic nucleus; VTA, ventral tegmental area; ZI, zona incerta.

Figure 8 shows an alignment of the amino acid sequence of rat GPR54 (SEQ.ID.NO.:4) with the amino acid sequence of rat GALR1 (SEQ.ID.NO.:5), rat GALR2 (SEQ.ID.NO.:6), rat GALR3 (SEQ.ID.NO.:7), and the rat opiod receptor DOR (SEQ.ID.NO.:8).

Figure 9 shows an alignment of the amino acid sequences of rat GPR54 (SEQ.ID.NO.:4) and human GPR54 (SEQ.ID.NO.:3).

30

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins.

35 Thus, a GPR54 protein preparation that is substantially free from other proteins will

contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-GPR54 proteins. Whether a given GPR54 protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

“Substantially free from other nucleic acids” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, a GPR54 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-GPR54 nucleic acids. Whether a given GPR54 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

“Functional equivalent” means a receptor which does not have exactly the same amino acid sequence as naturally occurring GPR54, due to alternative splicing, substitutions, deletions, mutations, or additions, but retains substantially the same biological activity as GPR54. Such functional equivalents will have significant amino acid sequence identity with naturally occurring GPR54. Genes and DNA encoding such functional equivalents can be detected by reduced stringency hybridization with a DNA sequence encoding naturally occurring GPR54. For the purposes of this invention, naturally occurring GPR54 has the amino acid shown as SEQ.ID.NO.:3 or SEQ.ID.NO.:4. A nucleic acid encoding a functional equivalent has at least about 50% identity at the nucleotide sequence level to SEQ.ID.NO.:1 or SEQ.ID.NO.:2.

A polypeptide has “substantially the same biological activity” as GPR54 if that polypeptide has a K_d for a ligand that is no more than 5-fold greater than the K_d of GPR54 having SEQ.ID.NO.:3 or SEQ.ID.NO.:4 for the same ligand. A polypeptide also has “substantially the same biological activity” as GPR54 if that polypeptide is capable of mediating the same functional response as naturally occurring GPR54 when exposed to the same ligand as naturally occurring GPR54. Examples of functional responses are: pigment aggregation in *Xenopus* melanophores,

changes in membrane currents in *Xenopus* oocytes, modulation of cAMP levels, changes in calcium concentration, changes in inositol phosphate levels, and coupling to inwardly rectifying potassium channels. One skilled in the art would be familiar with a variety of methods of measuring the functional responses of other G-protein coupled receptors and would be able to apply those methods to GPR54 (see, e.g., Lerner, 1994, Trends Neurosci. 17:142-146 [changes in pigment distribution in melanophore cells]; Yokomizo et al., 1997, Nature 387:620-624 [changes in cAMP or calcium concentration, chemotaxis]; Howard et al., 1996, Science 273:974-977 [changes in membrane currents in *Xenopus* oocytes]; McKee et al., 1997, Mol. Endocrinol. 11:415-423 [changes in calcium concentration measured using the aequorin assay]; Offermanns & Simon, 1995, J. Biol. Chem. 270:15175, 15180 [changes in inositol phosphate levels]). Zlokarnik et al., 1998, Science 279:84-88 and U.S. Patent No. 5,741,657 describe a reporter gene assay that can be adapted to measure GPR54 functional responses. The assay utilizes an inducible promoter-driven β -lactamase that cleaves a fluorescent substrate. Cleavage of the substrate leads to a change in fluorescence resonance energy transfer (FRET) between different portions of the substrate that is proportional to the magnitude of induction of the β -lactamase. Thus, the level of activation of the inducible promoter determines the amount of FRET measured. This level of induction of the promoter is in turn determined by the level of the substance (e.g., cAMP) the promoter is induced by. By choosing a promoter that is induced by a functional response that results from the interaction of a ligand and GPR54 (e.g., changes in cAMP levels), one can use this assay to measure GPR54 functional responses.

Depending upon the cells in which GPR54 is expressed, and thus the G-proteins with which GPR54 is coupled, certain of such methods as described above may be appropriate for measuring the functional responses of GPR54. It is well within the competence of one skilled in the art to select the appropriate method of measuring functional responses for a given experimental system.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid).

cDNA 1.2 Kb clone, named GPR54. Sequence analysis revealed the cloned GPR54 ORF to be identical to the previous phage clones and the original probe.

GPR54 contained an ORF of 1,185 bp encoding a protein of 395 amino acids.

5 Using GPR54 in a BLAST search (Altschul, 1997, Nucleic Acids Res 25:3389-3402), the highest identity was observed with the galanin and opioid receptor families. Specifically, GPR54 shared an amino acid sequence identity in the TM regions with rat galanin receptors GalR1(45%), GalR3 (45%), GalR2 (44%), and rat opioid receptor DOR (37%) (Figure 8). Conserved residues and consensus sequences
10 of the rhodopsin superfamily of GPCRs present in GPR54 included an asparagine in TM1, an aspartate in TM2, prolines in TMs 4 through 7, three consensus sequences for N-linked glycosylation in the amino terminus, cysteines in the first and second extracellular loops, a PKA/PKC consensus sequence in the second intracellular loop, a PKC consensus sequence in the third intracellular loop, and three possible
15 palmitoylation cysteine sites in the carboxy tail. Significantly, various residues in the human GalR1 receptor shown to be important for high-affinity galanin binding (corresponding to His262, His265, Glu269, and Phe280 in rat GalR1; (Kask et al., 1996, EMBO J. 15:236-244 (Kask); Berthold et al., 1997, Eur. J. Biochem. 249:601-606 (Berthold)) were not conserved in GPR54. Among these however, only His262 is
20 conserved among the three galanin receptors. In addition, the substitution of a tyrosine residue found in GPR54, GalR2 and GalR3 in place of Phe280 in GalR1 was shown to have no significant effect on galanin binding (Kask) as opposed to previous studies where Phe280 was replaced by alanine in GalR1 (Berthold).

Both Northern blot and *in situ* hybridization analyses of GPR54 were
25 performed at high stringencies and with a DNA probe encoding GPR54 from TM3 to TM7 and with low identities to the genes encoding galanin and related receptors. The tissue distribution of GPR54 was obtained by northern blot analysis using poly(A)⁺ RNA isolated from various rat tissues (Figure 6). In the brain, multiple RNA transcripts with a complex pattern were detected in the medulla pons, midbrain,
30 hippocampus, cortex, frontal cortex, and striatum. The most intense band was approximately 3.7 Kb in length, with a single, larger transcript of approximately 12 Kb length detected in the liver and intestine only. No transcripts were revealed in the cerebellum or kidney tissues.

Using *in situ* hybridization of rat brain sections, the distribution of
35 GPR54 mRNA was found to be discretely localized to many areas (Figure 7). The

highest levels of expression were seen in hypothalamic and amygdaloid nuclei. GPR54 mRNA was highly expressed in the zona incerta, ventral tegmental area, dentate gyrus, hypothalamic arcuate nucleus, dorsomedial hypothalamic nucleus, primary olfactory cortex, lateral habenular nucleus, lateral hypothalamic area, locus
5 coeruleus, and the cortical and medial nuclei of the amygdala. GPR54 mRNA was also concentrated in the superior colliculus, medial preoptic area, anterior hypothalamic area, posterior hypothalamic nucleus, periaqueductal gray, parafascicular thalamic nucleus, parabrachial nucleus, and ventral premammillary nucleus. The signals detected in the septohypothalamic nucleus, inferior colliculus,
10 medial nucleus of the amygdala, mesencephalic reticular nucleus and retrosplenial cortex were diffuse and less abundant.

GPR54's CNS expression pattern was found to resemble those of galanin receptors. Specifically, rat GalR1 mRNA expression is abundant in several brain regions including the hypothalamus, amygdala, hippocampus and locus
15 coeruleus (Parker et al., 1995, Mol. Brain Res. 34:179-189). Rat GalR2 mRNA expression is found in the mammillary nuclei, the dentate gyrus and posterior hypothalamic and arcuate nuclei (Kolakowski et al., 1998, J. Neurochem. 71, 2239-2251). Rat GalR3 is found to be abundantly expressed in the CA regions of Ammon's horn and the dentate gyrus with transcripts also detected in thalamic, hypothalamic,
20 mammillary and amygdaloid nuclei (Kolakowski et al., 1998, J. Neurochem. 71, 2239-2251).

The identity and overlapping expression patterns of GPR54 with the galanin receptors suggested that the encoded receptor may demonstrate binding to galanin. In preparation for expression and binding studies, the 1.2 kb cDNA fragment
25 encoding the ORF of GPR54 was subcloned into the multiple cloning site of the pcDNA3 expression vector and transiently transfected into COS-7 cells. No specific binding was observed with ¹²⁵I-human galanin. In contrast, specific and high affinity binding was observed under similar conditions with ¹²⁵I-human galanin in membranes prepared from COS cells transfected with human GalR1, consistent with a
30 previous report for GalR2 and GalR3 (Kolakowski et al., 1998, J. Neurochem. 71, 2239-2251).

A BLAST search with the rat GPR54 sequence revealed high identity with a human 3.5 Mb contig located in chromosome 19p13.3 containing a serine protease gene cluster (GenBank accession number AC005379). Sequence analysis
35 revealed a previously unrecognized 3.3 kb intron-containing human orthologue of

GPR54 encoding a protein 398 amino acids in length and sharing a translated amino acid identity of 81% (100% identity in the TM regions) with rat GPR54. The genomic sequence revealed four introns located in TM2 (~800 bp, interrupting the translated FYI..ANL sequence), TM3 (~800 bp, interrupting IQQ..VSV), TM4 (~250 bp, interrupting WVG..SAA) and in the third intracellular loop (~180 bp, interrupting ALQ..GQV).

One aspect of this invention is an isolated DNA comprising nucleotides encoding a polypeptide having the amino acid sequence SEQ.ID.NO.:3 or SEQ.ID.NO.:4. This isolated DNA can be substantially free from other nucleic acids and can be either single stranded or double stranded, *i.e.*, paired with its complementary sequence. Also within the present invention is isolated RNA corresponding to this DNA.

Another aspect of this invention is the identification and cloning of a cDNA which encodes GPR54, a G protein-coupled receptor. This cDNA is substantially free from other nucleic acids and can be either single stranded or double stranded. The present invention provides a cDNA molecule substantially free from other nucleic acids having the nucleotide sequence shown in Figure 1 as SEQ.ID.NO.:1 or in Figure 2 as SEQ.ID.NO.:2. SEQ.ID.NO.:1 contains an open reading frame (positions 1-1,194 of SEQ.ID.NO.:1) encoding a protein of 398 amino acids. SEQ.ID.NO.:2 contains an open reading frame (positions 61-1,245 of SEQ.ID.NO.:2) encoding a protein of 395 amino acids. (see Figure 5A-B).

Thus, the present invention also provides a DNA molecule substantially free from other nucleic acids comprising the nucleotide sequence of positions 1-1,194 of SEQ.ID.NO.:1 as well as a DNA molecule substantially free from other nucleic acids comprising the nucleotide sequence of positions 61-1,245 of SEQ.ID.NO.:2. The present invention also provides recombinant DNA molecules comprising the nucleotide sequence of positions 1-1,194 of SEQ.ID.NO.:1 or positions 61-1,245 of SEQ.ID.NO.:2.

Based on their predicted amino acid sequences, the human and rat GPR54 proteins most likely represent novel G-protein coupled receptors (GPCRs) since these GPR54 proteins obtain many of the characteristic features of GPCRs, *e.g.*.

- (a) seven transmembrane domains;
- (b) three intracellular loops;
- (c) three extracellular loops; and
- (d) the GPCR triplet signature sequence.

Northern blot and *in situ* hybridization analyses such as Figure 6 and Figure 7 showed that GPR54 RNA is widely expressed in rat brain regions (pons, midbrain, thalamus, hypothalamus, hippocampus, amygdala, cortex, frontal cortex, and striatum) as well as peripheral regions (liver and intestine).

5 The novel DNA sequences of the present invention encoding GPR54, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which GPR54 is not naturally linked, to form "recombinant DNA molecules" containing GPR54 sequences. The novel DNA sequences of the present invention can be inserted into vectors in order to direct recombinant expression of GPR54. Such
10 vectors may be comprised of DNA or RNA; for most purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode GPR54. One skilled in the art can readily determine an appropriate vector for a particular use.

15 Included in the present invention are DNA sequences that hybridize to SEQ.ID.NO.:1 or SEQ.ID.NO.:2 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured
20 salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

25 Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

30 Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the GPR54 protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2, but still encodes the same GPR54 protein as SEQ.ID.NO.:1 or SEQ.ID.NO.:2. Such synthetic DNAs are intended to be within the scope of the present invention. If it is desired to express such synthetic DNAs in a particular host cell or organism, the codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of that particular host cell or organism, thus leading to higher levels of expression of GPR54 protein in the host.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding GPR54. Such recombinant host cells can be cultured under suitable conditions to produce GPR54. An expression vector containing DNA encoding GPR54 can be used for expression of GPR54 in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey, and rodent origin, and insect cells including but not limited to, *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of GPR54 and which are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), HEK 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), *Xenopus* melanophores, and *Xenopus* oocytes.

Human embryonic kidney (HEK 293) cells and Chinese hamster ovary (CHO) cells are particularly suitable for expression of the GPR54 protein because these cells express a large number of G-proteins. Thus, it is likely that at least one of these G-proteins will be able to functionally couple the signal generated by interaction of GPR54 and its ligands, thus transmitting this signal to downstream effectors, eventually resulting in a measurable change in some assayable component, e.g., cAMP level, expression of a reporter gene, hydrolysis of inositol lipids, or intracellular Ca²⁺ levels.

Other cells that are particularly suitable for expression of the GPR54 protein are immortalized melanophore pigment cells from *Xenopus laevis*. Such melanophore pigment cells can be used for functional assays using recombinant expression of GPR54 in a manner similar to the use of such melanophore pigment cells for the functional assay of other recombinant GPCRs (Graminski et al., 1993, J. Biol. Chem. 268:5957-5964; Lerner, 1994, Trends Neurosci. 17:142-146; Potenza & Lerner, 1992, Pigment Cell Res. 5:372-378).

A variety of mammalian expression vectors can be used to express recombinant GPR54 in mammalian and other cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pCR2.1 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), pcDNA1 and pcDNA1amp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). For expression in non-mammalian cells, various suitable expression vectors are known in the art. The choice of vector will depend upon cell type used, level of expression desired, and the like. Following expression in recombinant cells, GPR54 can be purified to a level that is substantially free from other proteins by conventional techniques, e.g., salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography, hydrophobic interaction chromatography, and preparative gel electrophoresis.

The present invention includes GPR54 protein substantially free from other proteins. The amino acid sequence of the full-length human GPR54 protein is shown in Figure 3 as SEQ.ID.NO.:3. The amino acid sequence of the full-length rat GPR54 protein is shown in Figure 4 as SEQ.ID.NO.:4. Thus, the present invention includes GPR54 proteins substantially free from other proteins having the amino acid sequence of SEQ.ID.NO.:3 or SEQ.ID.NO.:4.

As with many receptor proteins, it is possible to modify many of the amino acids of GPR54, particularly those which are not found in the ligand binding domain, and still retain substantially the same biological activity as the original receptor. Thus, the present invention includes modified GPR54 polypeptides which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as naturally occurring GPR54. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., Molecular Biology of the Gene, Watson et al., 1987, Fourth Ed.,

The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:3 or SEQ.ID.NO.:4 wherein the polypeptides still retain substantially the same biological activity as naturally occurring GPR54. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:3 or SEQ.ID.NO.:4 wherein the polypeptides still retain substantially the same biological activity as naturally occurring GPR54. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the ligand-binding domain of GPR54.

When deciding which amino acid residues of GPR54 may be substituted to produce polypeptides that are functional equivalents of GPR54, one skilled in the art would be guided by a comparison of the amino acid sequence of GPR54 with the amino acid sequences of related proteins, *e.g.*, the human, mouse, or rat GALR1, GALR2, or GALR3 receptors, as well as the rat opiod receptor DOR (see, *e.g.*, Figure 8). Such a comparison would allow one skilled in the art to minimize the number of amino acid substitutions made in regions that are highly conserved between GPR54 and the related proteins. Accordingly, the present invention includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:3 or SEQ.ID.NO.:4 where the polypeptides still retain substantially the same biological activity as naturally occurring GPR54 and where the substitutions are conservative and do not occur in positions where GPR54 and any of the human, mouse, or rat GALR1, GALR2, or GALR3 receptors share the same amino acid, or do not occur in positions where GPR54 and the rat opiod DOR receptor share the same amino acid (see Figure 8). In particular embodiments, the substitutions do not occur in positions where GPR54 and any of the rat GALR1, GALR2, or GALR3 receptors share the same amino acid (see Figure 8).

One skilled in the art would also recognize that polypeptides that are functional equivalents of GPR54 and have changes from the GPR54 amino acid sequence that are small deletions or insertions of amino acids could also be produced by following the same guidelines, *i.e.*, minimizing the differences in amino acid sequence between GPR54 and related proteins. Small deletions or insertions are generally in the range of about 1 to 5 amino acids. The effect of such small deletions

or insertions on the biological activity of the modified GPR54 polypeptide can easily be assayed by producing the polypeptide synthetically or by making the required changes in DNA encoding GPR54 and then expressing the DNA recombinantly and assaying the protein produced synthetically or by such recombinant expression.

- 5 Assays that could be used include simple binding assays to determine if the modified GPR54 polypeptide is capable of binding the same ligands, with approximately the same affinity, as naturally occurring GPR54 protein. Alternatively, one can use functional assays such as assays such as those described herein.

- 10 The present invention also includes C-terminal truncated forms of GPR54, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor. Such truncated receptors are useful in various binding assays described herein, for crystallization studies, and for structure-activity-relationship studies.

- 15 The present invention also includes chimeric GPR54 proteins. Chimeric GPR54 proteins consist of a contiguous polypeptide sequence of GPR54 fused in frame to a polypeptide sequence of a non-GPR54 protein. For example, the N-terminal domain and seven transmembrane spanning domains of GPR54 fused at the C-terminus in frame to a G protein would be a chimeric GPR54 protein.

- 20 The present invention also includes GPR54 proteins that are in the form of multimeric structures, *e.g.*, dimers. Such multimers of other G-protein coupled receptors are known (Hebert *et al.*, 1996, J. Biol. Chem. 271, 16384-16392; Ng *et al.*, 1996, Biochem. Biophys. Res. Comm. 227, 200-204; Romano *et al.*, 1996, J. Biol. Chem. 271, 28612-28616). The dimers may be homodimers containing two GPR54 proteins or the dimers may be heterodimers containing GPR54 and another
25 protein.

The present invention also includes isolated forms of GPR54 proteins.

- 30 The present invention includes methods of identifying compounds that specifically bind to GPR54 protein, as well as compounds identified by such methods. The specificity of binding of compounds having affinity for GPR54 is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from such cells. Expression of the cloned receptor and screening for compounds that bind to GPR54, or that inhibit the binding of a known ligand of GPR54 to such cells, or membranes prepared from such cells, provides an effective method for the rapid selection of compounds with high affinity for GPR54.
35 Such ligands or compounds can be radiolabeled, but can also be nonisotopic

compounds that can be used to displace bound radiolabeled ligands or that can be used as activators or inhibitors in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of GPR54 and may be peptides, proteins, or non-proteinaceous organic molecules. Such compounds are likely to be pharmacologically useful modulators of GPR54 activity.

Therefore, the present invention includes assays by which GPR54 agonists and antagonists may be identified. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of GPR54. Accordingly, the present invention includes a method for determining whether a substance is a potential agonist or antagonist of GPR54 that comprises:

- (a) transfecting cells with an expression vector encoding GPR54;
 - (b) allowing the transfected cells to grow for a time sufficient to allow GPR54 to be expressed;
 - (c) exposing the cells to a labeled ligand of GPR54 in the presence and in the absence of the substance;
 - (d) measuring the binding of the labeled ligand to GPR54;
- where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of GPR54.

The conditions under which step (c) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

The present invention also includes a method for determining whether a substance is capable of binding to GPR54, *i.e.*, whether the substance is a potential agonist or an antagonist of GPR54, where the method comprises:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of GPR54 in the cells;
- (b) exposing the test cells to the substance;
- (c) measuring the amount of binding of the substance to GPR54 in the test cells;

(d) comparing the amount of binding of the substance to GPR54 in the test cells with the amount of binding of the substance to control cells that have not been transfected with GPR54;

5 wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to GPR54. Determining whether the substance is an agonist or antagonist can then be accomplished by the use of functional assays such as, *e.g.*, the assay involving the use of promiscuous G-proteins described below.

10 The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

15 In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), HEK 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2),
20 C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

The assays described above can be carried out with cells that have been transiently or stably transfected with GPR54. Transfection is meant to include any method known in the art for introducing GPR54 into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection,
25 lipofection, infection with a retroviral construct containing GPR54, and electroporation.

Where binding of the substance or ligand to GPR54 is measured, such binding can be measured by employing a labeled substance or ligand. The substance or ligand can be labeled in any convenient manner known to the art, *e.g.*,
30 radioactively, fluorescently, enzymatically.

In particular embodiments of the above-described methods, GPR54 has an amino acid sequence of SEQ.ID.NO.:3 or SEQ.ID.NO.:4.

The above-described methods can be modified in that, rather than exposing the test cells to the substance, membranes can be prepared from the test cells
35 and those membranes can be exposed to the substance. Such a modification utilizing

membranes rather than cells is well known in the art and is described in, *e.g.*, Hess *et al.*, 1992, Biochem. Biophys. Res. Comm. 184:260-268.

Accordingly, the present invention provides a method for determining whether a substance is capable of binding to GPR54 comprising:

- 5 (a) providing test cells by transfecting cells with an expression vector that directs the expression of GPR54 in the cells;
- (b) preparing membranes containing GPR54 from the test cells and exposing the membranes to a ligand of GPR54 under conditions such that the ligand binds to the GPR54 in the membranes;
- 10 (c) subsequently or concurrently to step (b), exposing the membranes from the test cells to a substance;
- (d) measuring the amount of binding of the ligand to the GPR54 in the membranes in the presence and the absence of the substance;
- (e) comparing the amount of binding of the ligand to GPR54 in the
15 membranes in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to GPR54 in the membranes in the presence of the substance indicates that the substance is capable of binding to GPR54.

In particular embodiments, GPR54 has an amino acid sequence of SEQ.ID.NO.:3 or SEQ.ID.NO.:4.

- 20 The present invention provides a method for determining whether a substance is capable of binding to GPR54 comprising:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of GPR54 in the cells;
 - (b) preparing membranes containing GPR54 from the test cells and
25 exposing the membranes from the test cells to the substance;
 - (c) measuring the amount of binding of the substance to the GPR54 in the membranes from the test cells;
 - (d) comparing the amount of binding of the substance to GPR54 in the membranes from the test cells with the amount of binding of the substance to
30 membranes from control cells that have not been transfected with GPR54;
- where if the amount of binding of the substance to GPR54 in the membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to GPR54.

In particular embodiments, GPR54 has an amino acid sequence of SEQ.ID.NO.:3 or SEQ.ID.NO.:4.

As a further modification of the above-described methods, RNA encoding GPR54 can be prepared, *e.g.*, by *in vitro* transcription using a plasmid containing GPR54 under the control of a bacteriophage T7 promoter, and the RNA can be microinjected into *Xenopus* oocytes in order to cause the expression of GPR54 in the oocytes. Substances are then tested for binding to the GPR54 expressed in the oocytes. Alternatively, rather than detecting binding, the effect of the substances on the electrophysiological properties of the oocytes can be determined.

The present invention includes assays by which GPR54 agonists and antagonists may be identified by their ability to stimulate or antagonize a functional response mediated by GPR54. One skilled in the art would be familiar with a variety of methods of measuring the functional responses of G-protein coupled receptors (see, *e.g.*, Lerner, 1994, Trends Neurosci. 17:142-146 [changes in pigment distribution in melanophore cells]; Yokomizo et al., 1997, Nature 387:620-624 [changes in cAMP or calcium concentration; chemotaxis]; Howard et al., 1996, Science 273:974-977 [changes in membrane currents in *Xenopus* oocytes]; McKee et al., 1997, Mol. Endocrinol. 11:415-423 [changes in calcium concentration measured using the aequorin assay]; Offermanns & Simon, 1995, J. Biol. Chem. 270:15175, 15180 [changes in inositol phosphate levels]).

Accordingly, the present invention provides a method of identifying agonists and antagonists of GPR54 comprising:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of GPR54 in the cells;
 - (b) exposing the test cells to a substance that is suspected of being an agonist or an antagonist of GPR54;
 - (c) measuring the amount of a functional response of the test cells that have been exposed to the substance;
 - (d) comparing the amount of the functional response exhibited by the test cells with the amount of the functional response exhibited by control cells;
- wherein if the amount of the functional response exhibited by the test cells differs from the amount of the functional response exhibited by the control cells, the substance is an agonist or antagonist of GPR54;

where the control cells are cells that have not been transfected with GPR54 but have been exposed to the substance or are test cells that have not been exposed to the substance.

In particular embodiments, GPR54 has an amino acid sequence of
5 SEQ.ID.NO.:3 or SEQ.ID.NO.:4.

In particular embodiments, the functional response is selected from the group consisting of: changes in pigment distribution in melanophore cells; changes in cAMP or calcium concentration; changes in membrane currents in *Xenopus* oocytes; and changes in inositol phosphate levels.

10 GPR54 belongs to the class of proteins known as G-protein coupled receptors (GPCRs). GPCRs transmit signals across cell membranes upon the binding of ligand. The ligand-bound GPCR interacts with a heterotrimeric G-protein, causing the $G\alpha$ subunit of the G-protein to disassociate from the $G\beta$ and $G\gamma$ subunits. The $G\alpha$ subunit can then go on to activate a variety of second messenger systems.

15 Generally, a particular GPCR is only coupled to a particular type of G-protein. Thus, to observe a functional response from the GPCR, it is necessary to ensure that the proper G-protein is present in the system containing the GPCR. It has been found, however, that there are certain G-proteins that are "promiscuous." These promiscuous G-proteins will couple to, and thus transduce a functional signal from,
20 virtually any GPCR. See Offermanns & Simon, 1995, J. Biol. Chem. 270:15175, 15180 (Offermanns). Offermanns described a system in which cells are transfected with expression vectors that result in the expression of one of a large number of GPCRs as well as the expression of one of the promiscuous G-proteins $G\alpha 15$ or $G\alpha 16$. Upon the addition of an agonist of the GPCR to the transfected cells, the
25 GPCR was activated and was able, via $G\alpha 15$ or $G\alpha 16$, to activate the β isoform of phospholipase C, leading to an increase in inositol phosphate levels in the cells.

Therefore, by making use of these promiscuous G-proteins as in Offermanns, it is possible to set up functional assays for GPR54, even in the absence of knowledge of the G-protein with which GPR54 is coupled *in vivo*. One possibility
30 is to create a fusion or chimeric protein composed of the extracellular and membrane spanning portion of GPR54 fused to a promiscuous G-protein. Such a fusion protein would be expected to transduce a signal following binding of ligand to the GPR54 portion of the fusion protein. Accordingly, the present invention provides a method of identifying antagonists of GPR54 comprising:

- (a) providing cells that expresses a chimeric GPR54 protein fused at its C-terminus to a promiscuous G-protein;
- (b) exposing the cells to an agonist of GPR54;
- (c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of GPR54;

5 (d) measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of GPR54.

10 Another possibility for utilizing promiscuous G-proteins in connection with GPR54 includes a method of identifying agonists of GPR54 comprising:

- (a) providing cells that expresses both GPR54 and a promiscuous G-protein;
- (b) exposing the cells to a substance that is a suspected agonist of GPR54;

15 (c) measuring the level of inositol phosphates in the cells; where an increase in the level of inositol phosphates in the cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist indicates that the substance is an agonist of GPR54.

20 Levels of inositol phosphates can be measured by monitoring calcium mobilization. Intracellular calcium mobilization is typically assayed in whole cells under a microscope using fluorescent dyes or in cell suspensions via luminescence using the aequorin assay.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), *Xenopus* oocytes, or *Xenopus* melanophores.

25 In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of GPR54 and the promiscuous G-protein in the cells.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about
5 55°C.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16. Expression vectors containing Gα15 or Gα16 are known in the art. See, *e.g.*, Offermanns; Buhl *et al.*, 1993, FEBS Lett. 323:132-134; Amatruda *et al.*, 1993, J.
10 Biol. Chem. 268:10139-10144.

The above-described assay can be modified to form a method to identify antagonists of GPR54. Such a method is also part of the present invention and comprises:

- (a) providing cells that expresses both GPR54 and a promiscuous
15 G-protein;
- (b) exposing the cells to a substance that is an agonist of GPR54;
- (c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of GPR54;
- (d) measuring the level of inositol phosphates in the cells;
- 20 where a decrease in the level of inositol phosphates in the cells in the presence of the suspected antagonist as compared to the level of inositol phosphates in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of GPR54.

In a particular embodiment of the above-described method, the cells
25 are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271
30 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), *Xenopus* oocytes, or *Xenopus* melanophores.

The conditions under which steps (b) and (c) of the method are practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such

commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

5 In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of GPR54 and the promiscuous G-protein in the cells.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16.

In particular embodiments of the above-described methods, GPR54 has an amino acid sequence of SEQ.ID.NO.:3 or SEQ.ID.NO.:4.

10 While the above-described methods are explicitly directed to testing whether “a” substance is an agonist or antagonist of GPR54, it will be clear to one skilled in the art that such methods can be adapted to test collections of substances, *e.g.*, combinatorial libraries, to determine whether any members of such collections are activators or inhibitors of GPR54. Accordingly, the use of collections of
15 substances, or individual members of such collections, as the substance in the above-described methods is within the scope of the present invention.

Agonists and antagonists of GPR54 that are identified by the above-described methods are expected to have utility in the treatment of diseases that involve the inappropriate expression of GPR54. In particular, given the resemblance
20 between GPR54 and the galanin receptors, it is expected that agonists and antagonists of GPR54 will have pharmacological activity and be useful in a manner similar to that in which agonists and antagonists of the galanin receptors are useful. Therefore, agonists and antagonists of GPR54 are expected to be useful in the treatment of: eating disorders and obesity; Alzheimer’s disease and other disorders affecting
25 memory; pain; sexual disorders; and growth hormone imbalances.

The present invention includes pharmaceutical compositions comprising agonists and antagonists of GPR54. The agonists and antagonists are generally combined with pharmaceutically acceptable carriers to form pharmaceutical compositions. Examples of such carriers and methods of formulation of
30 pharmaceutical compositions containing agonists and antagonists and carriers can be found in Remington’s Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain a therapeutically effective amount of the agonists and antagonists.

The present invention also includes methods of expressing GPR54 in recombinant systems and then utilizing the recombinantly expressed GPR54 receptor protein for counter-screening. When screening compounds in order to identify potential pharmaceuticals that specifically interact with a target receptor, it is
5 necessary to ensure that the compounds identified are as specific as possible for the target receptor. To do this, it is necessary to screen the compounds against as wide an array as possible of receptors that are similar to the target receptor. Thus, in order to find compounds that are potential pharmaceuticals that interact with receptor A, it is necessary not only to ensure that the compounds interact with receptor A (the "plus
10 target") and produce the desired pharmacological effect through receptor A, it is also necessary to determine that the compounds do not interact with receptors B, C, D, *etc.* (the "minus targets"). In general, as part of a screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, *Bio/Technology* 10:973-980, at 980). Therefore, GPR54 proteins and DNA encoding GPR54 proteins have
15 utility in counter-screens. That is, they can be used as "minus targets" in counter-screens in connection with screening projects designed to identify compounds that specifically interact with other G-protein coupled receptors.

The DNA of the present invention, or hybridization probes based upon the DNA, can be used in chromosomal mapping studies in order to identify the precise
20 chromosomal location of the GPR54 gene or of genes encoding proteins related to GPR54. While the present inventors have determined that the human GPR54 gene is located at chromosome 19p13.3, it may be desirable to perform mapping studies to even more precisely locate the human GPR54 gene. Such mapping studies can be carried out using well-known genetic and/or chromosomal mapping techniques such
25 as, *e.g.*, linkage analysis with respect to known chromosomal markers or *in situ* hybridization. See, *e.g.*, Verma et al., 1988, Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY. After identifying the precise chromosomal location of the GPR54 gene or genes encoding proteins related to GPR54, this information can be compared with the locations of known disease-
30 causing genes contained in genetic map data (such as the data found in the genome issue of *Science* (1994, 265:1981-2144). In this way, one can correlate the chromosomal location of the GPR54 gene or of genes encoding proteins related to GPR54 with the locations of known disease-causing genes and thus help to limit the region of DNA containing such disease-causing genes. This will simplify the process
35 of cloning such disease-causing genes. Also, once linkage between the precise

chromosomal location of the GPR54 gene or of genes encoding proteins related to GPR54 and the locations of a known disease-causing gene is established, that linkage can be used diagnostically to identify restriction fragment length polymorphisms (RFLPs) in the vicinity of the GPR54 gene or of genes encoding proteins related to GPR54. Such RFLPs will be associated with the disease-causing gene and thus can be used to identify individuals carrying the disease-causing gene.

For such chromosomal mapping studies as described herein, it may be advantageous to use, in addition to the DNA of the present invention, the reverse complement of the DNA of the present invention or RNA corresponding to the DNA of the present invention.

Nucleotide sequences that are complementary to the GPR54 sequences disclosed herein can be synthesized for use in antisense therapy. Such antisense molecules can be DNA, stable derivatives of DNA such as phosphorothioates or methyl phosphonates, RNA, stable derivatives of RNA such as 2'-O-alkyl RNA, or other forms of GPR54 antisense molecules. GPR54 antisense molecules can be introduced into cells by a variety of methods, *e.g.*, microinjection, liposome encapsulation, or by expression from vectors harboring the antisense sequence. GPR54 antisense therapy is expected to be particularly useful in the treatment of conditions where it is beneficial to reduce GPR54 activity.

The present invention also includes antibodies to the GPR54 protein. Such antibodies may be polyclonal antibodies or monoclonal antibodies and are useful in treating disorders that involve the inappropriate expression or activity of the GPR54 protein. The antibodies of the present invention are raised against the entire GPR54 protein or against suitable antigenic fragments of the protein that are coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, GPR54 protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, GPR54 protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells
5 are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce GPR54 polypeptides into the
10 cells of target organs. Nucleotides encoding GPR54 polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding GPR54 polypeptides can be transferred into cells for gene therapy by non-
15 viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with GPR54 polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate GPR54 activity.

20 A cDNA fragment encoding full-length GPR54 can be isolated from an appropriate human cDNA library by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the cDNA sequence for GPR54 shown in Figure 1 as SEQ.ID.NO.:1. Suitable primer pairs would be, *e.g.*:

25
5'-ATG CAC ACC GTG GCT ACG TCC-3' (SEQ.ID.NO.:11) and
5'-TCA GAG AGG GGC GTT GTC CTC-3' (SEQ.ID.NO.:12).

The above primers may contain restriction sites in their 5' ends to
30 facilitate cloning of the amplified cDNA into suitable vectors, *e.g.*, pcDNA3.1. The above primers are meant to be illustrative. One skilled in the art would recognize that a variety of other suitable primers can be designed.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase.
35 For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM

MgCl₂, 200 µM for each dNTP, 50 mM KCl, 0.2 µM for each primer, 10 ng of DNA template, 0.05 units/µl of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press.

A suitable cDNA library from which a clone encoding GPR54 can be isolated would be a human cDNA library made from RNA from brain tissue. Such libraries can be prepared by methods well-known in the art. Alternatively, several commercially available libraries would be suitable, *e.g.*, cDNA libraries such as human fetal brain, catalog #937227 from Stratagene, Inc., La Jolla, CA, USA, and human brain hypothalamus, catalog #HL1172a, from Clontech Laboratories, Inc., Palo Alto, CA, USA. The primary clones of such libraries can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified separately.

By this method, a cDNA fragment encoding an open reading frame of 398 amino acids (SEQ.ID.NO.:3) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, Ca). GPR54 protein can then be produced by transferring an expression vector encoding GPR54 into suitable host cells and growing the host cells under appropriate conditions. GPR54 protein can then be isolated by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone encoding GPR54 can be isolated from a cDNA library using as a probe oligonucleotides specific for GPR54 and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described in, *e.g.*, Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II. Oligonucleotides that are specific for GPR54 and that can be used to screen cDNA libraries can be readily designed based upon the cDNA sequence of GPR54 shown in Figure 1 as SEQ.ID.NO.:1 and can be synthesized by methods well-known in the art.

Genomic clones containing the GPR54 gene can be obtained from commercially available human PAC or BAC libraries, *e.g.*, from Research Genetics, Huntsville, AL. Alternatively, one may prepare genomic libraries, for example in P1 artificial chromosome vectors, from which genomic clones containing the GPR54 can be isolated, using probes based upon the GPR54 nucleotide sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou *et al.*, 1994, Nature Genet. 6:84-89).

The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

PCR amplification and cDNA library screening

A rat brain 5' Stretch cDNA library (Clontech) was amplified by the polymerase chain reaction (PCR) using proof-reading *Pfu* polymerase (Stratagene) and degenerate oligonucleotides based upon sequences encoding GPCR conserved transmembrane (TM) region 3

P1: 5'-CTGACCGGCATGABDETFGADCGHTA-3' (SEQ.ID.NO.:9)

and transmembrane (TM) region 7

P2: 5'-GAAGGCGTAGAFBAIJGGKTT)-3' (SEQ.ID.NO.:10)

where B = C or G, D = C or T, E = A or G or T, F = C or G or T, H = A or C, I = A or C or G or T, J = A or C or G, K = A or G.

PCR conditions were as follows: denaturation at 94°C for 30 sec, annealing at 55, 48, 45, 42, or 40°C for 40 sec, and extension at 72°C for 30 sec, for 30 cycles, followed by a 7 min extension at 72°C. The PCR products were extracted with phenol/chloroform, precipitated with ethanol and electrophoresed on a low melting point agarose gel. PCR product bands in the expected size range were excised from the gel, ligated into the *EcoRV* site of pBluescript SK(-) (Stratagene)

and sequenced. One insert appeared to encode a novel GPCR and was labeled with [32P] dCTP- α (NEN) by nick translation (Amersham) and used to screen the same library amplified above as previously described (Marchese et al., 1994, Genomics 23:609-618). Positive phage clones were plaque purified and their inserts amplified
5 by PCR using *Pfu* polymerase and primers flanking the *lgt11 EcoRI* cloning site. The PCR products were blunt-end ligated into the *EcoRV* site of pBluescript SK(-) (Stratagene) and sequenced on both strands.

EXAMPLE 2

10 Northern blot analysis

Rat mRNAs from several rat tissues were extracted as described previously (Marchese et al., 1994, Genomics 23:609-618). Briefly, total RNA was extracted by the method of Chomczynski & Sacchi, 1987, Anal. Biochem. 162:156-159 and poly (A)⁺ RNA isolated using oligo(dT) cellulose spin columns (Pharmacia,
15 Uppsala, Sweden). RNA was denatured and size fractionated on a 1% formaldehyde agarose gel, transferred onto nylon membrane and immobilized by UV irradiation. The blots were hybridized with a ³²P-labeled DNA fragment encoding GPR54, washed with 2X SSPE and 0.1% SDS at 50°C for 20 min and again with 0.1X SSPE and 0.1% SDS at 50°C for 2 h and exposed to X-ray film at -70°C in the presence of
20 an intensifying screen.

EXAMPLE 3

In situ hybridization analysis

An ³⁵S-labeled DNA fragment encoding GPR54 was used as a probe
25 for *in situ* hybridization. Preparation of rat brain sections and *in situ* hybridization procedures were done as previously described (O'Dowd et al., 1996, FEBS Lett 394:325-329).

EXAMPLE 4

Expression of GPR54 cDNA in COS-7 mammalian cells

The African Green Monkey SV40 transformed kidney cell line (COS-7 cells), obtained from the American Type Culture Collection, was grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum (Sigma), 50 units/ml penicillin, 50 µg/ml streptomycin (Flow Laboratories, McLean, VA), and 2 mM glutamine (Flow Laboratories) at 37°C under an atmosphere of 6% CO₂. 5 X 10⁶ cells per 175- cm² culture flask were seeded in 20 ml of media and transiently transfected at 80% confluence with either 2.75, 5.5, or 11.65 µg of pcDNA3-GPR54 or pcIneo-hGALR1 plasmids and 70 µl of LipofectAMINE reagent (Life Technologies, Inc.), following recommendations of the manufacturer. Two days after transfection, cells were harvested following dissociation in enzyme-free dissociation solution (Specialty Media, Lavallette, NJ).

15 EXAMPLE 5

Membrane preparation and radioligand binding assays

Membranes were prepared from transfected cells by disruption by pressurized nitrogen cavitation in ice-cold membrane buffer (10 mM Tris, pH 7.4, 10 mM phenylmethylsulfonylfluoride, 10 mM phosphoramidon). After a low speed (1100 x g for 10 min. at 4°C) and a high speed centrifugation (38,700 x g for 15 min. at 4°C), membranes were resuspended in buffer and their protein concentration determined (Bio-Rad assay kit). Binding of ¹²⁵I-human galanin (specific activity of 2200 Ci/mmol, DuPont NEN) was measured in membranes using a buffer of 25 mM Tris, pH 7.4, 0.3% BSA, 2 mM MgCl₂, 4 mg/ml phosphoramidon, and 10 mM leupeptin in a total volume of 250 µl. 200 pM of ¹²⁵I-human galanin was used. Reactions were initiated by the addition of membranes and the incubation was allowed to proceed at room temperature for 2 hours. Non-specific binding was defined as the amount of radioactivity remaining bound in the presence of 10 mM unlabeled human galanin. Incubations were terminated by rapid filtration through GF/C filters which had been presoaked with 0.1% polyethylamine using a TOMTEC (Orange, CT) cell harvester.

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED:

1. An isolated DNA comprising nucleotides encoding a polypeptide having the amino acid sequence SEQ.ID.NO.:3 or SEQ.ID.NO.:4.
- 5 2. The DNA molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of SEQ.ID.NO.:1; positions 1-1,194 of SEQ.ID.NO.:1; SEQ.ID.NO.:2; and positions 61-1,245 of SEQ.ID.NO.:2.
- 10 3. A DNA molecule that hybridizes under stringent conditions to the DNA of claim 1.
4. An expression vector comprising the DNA of claim 1.
- 15 5. A recombinant host cell comprising the DNA of claim 1.
6. An isolated polypeptide comprising a GPR54 protein having the amino acid sequence SEQ.ID.NO.:3 or SEQ.ID.NO.:4
- 20 7. The isolated polypeptide of claim 6 that is substantially free from other proteins.
8. The isolated polypeptide of claim 6 containing a single amino acid substitution.
- 25 9. The isolated polypeptide of claim 6 containing two or more amino acid substitutions where the substitutions are conservative and do not occur in positions where GPR54 and any of the rat GALR1, GALR2, or GALR3 receptors share the same amino acid.
- 30 10. A method for determining whether a substance is an agonist or antagonist of GPR54 comprising:
 - (a) transfecting cells with an expression vector encoding GPR54;

- (b) allowing the transfected cells to grow for a time sufficient to allow GPR54 to be expressed;
- (c) exposing the cells to a labeled ligand of GPR54 in the presence and in the absence of the substance;
- 5 (d) measuring the binding of the labeled ligand to GPR54; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is an agonist or antagonist of GPR54;
- 10 where GPR54 has the amino acid sequence SEQ.ID.NO.:3 or SEQ.ID.NO.:4.

11. A method for determining whether a substance is capable of binding to GPR54 comprising:
- (a) providing test cells by transfecting cells with an expression
15 vector that directs the expression of GPR54 in the cells;
- (b) exposing the test cells to the substance;
- (c) measuring the amount of binding of the substance to GPR54 in the test cells;
- (d) comparing the amount of binding of the substance to GPR54 in
20 the test cells with the amount of binding of the substance to control cells that have not been transfected with GPR54;
- wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to GPR54;
- where GPR54 has the amino acid sequence SEQ.ID.NO.:3 or
25 SEQ.ID.NO.:4.

12. A method of identifying agonists and antagonists of GPR54 comprising:
- (a) providing test cells by transfecting cells with an expression
30 vector that directs the expression of GPR54 in the cells;
- (b) exposing the test cells to a substance that is suspected of being an agonist or an antagonist of GPR54;
- (c) measuring the amount of a functional response of the test cells that have been exposed to the substance;

- (d) comparing the amount of the functional response exhibited by the test cells with the amount of the functional response exhibited by control cells;
wherein if the amount of the functional response exhibited by the test cells differs from the amount of the functional response exhibited by the control cells,
5 the substance is an agonist or antagonist of GPR54;
where the control cells are cells that have not been transfected with GPR54 but have been exposed to the substance or are test cells that have not been exposed to the substance;
where GPR54 has the amino acid sequence SEQ.ID.NO.:3 or
10 SEQ.ID.NO.:4.

13. An antibody that binds specifically to GPR54 where GPR54 has the amino acid sequence SEQ.ID.NO.:3 or SEQ.ID.NO.:4.

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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: G PROTEIN-COUPLED RECEPTOR RESEMBLING GALANIN RECEPTORS

(57) Abstract: Human and rat DNAs encoding a novel G-protein coupled receptor, GPR54, as well as proteins encoded by the DNAs, are provided. Methods of identifying agonists and antagonists of GPR54 are also provided.

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Met His Thr Val Ala Thr Ser Gly Pro Asn Ala Ser Trp Gly Ala Pro Ala Asn Ala
 ATG CAC ACC GTG GCT ACG TCC GGA CCC AAC GCG TCC TGG GGG GCA CCG GCC AAC GCC
 9 18 27 36 45 54

Ser Gly Cys Pro Gly Cys Gly Ala Asn Ala Ser Asp Gly Pro Val Pro Ser Pro Arg Ala Val
 TCC GGC TGC CCG GGC TGT GGC GCC AAC GCC TCG GAC GGC CCA GTC CCT TCG CCG CCG GCC GTG
 66 75 84 93 102 111 120

Asp Ala Trp Leu Val Pro Leu Phe Phe Ala Ala Leu Met Leu Leu Gly Leu Val Gly Asn Ser
 GAC GCC TGG CTC GTG CCG CTC TTC TTC GCG GCG CTC ATG CTG CTG GGC CTG GTG GCG AAC TCG
 129 138 147 156 165 174 183

Leu Val Ile Tyr Val Ile Cys Arg His Lys Pro Met Arg Thr Val Thr Asn Phe Tyr Ile Ala
 CTG GTC ATC TAC GTC ATC TGC CCG CAC AAG CCG ATG CCG ACC GTG ACC AAC TTC TAC ATC GCC
 192 201 210 219 228 237 246

Asn Leu Ala Ala Thr Asp Val Thr Phe Leu Leu Cys Cys Val Pro Phe Thr Ala Leu Leu Tyr
 AAC CTG GCG GCC ACG GAC GTG ACC TTC CTC CTC TCG TGC GTC GTC CCC TTC ACG GCC CTG CTG TAC
 225 264 273 282 291 300 309

Pro Leu Pro Gly Trp Val Leu Gly Asp Phe Met Cys Lys Phe Val Asn Tyr Ile Gln Gln Val
 CCG CTG CCC GGC TGG GTG CTG GGC GAC TTC ATG TGC AAG TTC GTC AAC TAC ATC CAG CAG GTC
 318 327 336 345 354 363 372

FIG.1A

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Ser Val Gln Ala Thr Cys Ala Thr Leu Thr Ala Met Ser Val Asp Arg Trp Tyr Val Thr Val
 TCG GTG CAG GCC ACC TGT GCC ACT CTG ACC GCC ATG AGT GTG GAC CGC TGG TAC GTG ACG GTG
 381 390 399 408 417 426 435

 Phe Pro Leu Arg Ala Leu His Arg Arg Thr Pro Arg Leu Ala Leu Val Ser Leu Ser Ile
 TTC CCG TTG CGC GCC CTG CAC CGC CGC ACC CCC CGC CTG GCT GTG AGC CTC AGC ATC
 444 453 462 471 480 489 498

 Trp Val Gly Ser Ala Ala Val Ser Ala Pro Val Leu Ala Leu His Arg Leu Ser Pro Gly Pro
 TGG GTA GGC TCT GCG GCG GTG TCT GCG CCG GTG CTC GCC CTG CAC CGC CTG TCA CCC GCG CCG
 507 516 525 534 543 552 561

 Arg Ala Tyr Cys Ser Glu Ala Phe Pro Ser Arg Ala Leu Glu Arg Ala Phe Ala Leu Tyr Asn
 CGC GCC TAC TGC AGT GAG GCC TTC CCC AGC CGC GCC CTG GAG CGC GCC TTC GCA CTG TAC AAC
 570 579 588 597 606 615 624

 Leu Leu Ala Leu Tyr Leu Leu Pro Leu Leu Thr Cys Ala Cys Tyr Ala Ala Met Leu Arg
 CTG CTG GCG CTG TAC CTG CTG CCG CTG CTC GCC ACC TGC GCC TGC TAT GCG GCC ATG CTG CGC
 633 642 651 660 669 678 687

 His Leu Gly Arg Val Ala Val Arg Pro Ala Pro Ala Asp Ser Ala Leu Gln Gly Gln Val Leu
 CAC CTG GGC CGG GTC GCC GTG CCG CCC GCG CCC GAT AGC GCC CTG CAG GCG CAG GTG CTG
 696 705 714 723 732 741 750

 Ala Glu Arg Ala Gly Ala Val Arg Ala Lys Val Ser Arg Leu Val Ala Ala Val Val Leu Leu
 GCA GAG CCG GCA GGC GCC GTG CCG GCG AAC GTC TCG CCG CTG GTG GCC GCC GTG CTC CTC
 759 768 777 786 795 804 813

FIG.1B

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Phe Ala Ala Cys Trp Gly Pro Ile Gln Leu Phe Leu Val Leu Gln Ala Leu Gly Pro Ala Gly
TTC GCC GCC TGC TGG GGC CCC ATC CAG CTG TTC CTG CTG GTG CTG CAG GCG CTG GCG CCC GCG GCG
822 831 840 849 858 867 876

Ser Trp His Pro Arg Ser Tyr Ala Ala Tyr Ala Leu Lys Thr Trp Ala His Cys Met Ser Tyr
TCC TGG CAC CCA CCG AGC TAC GCC GCC TAC GCG CTT AAG ACC TGG GCT CAC TGC ATG TCC TAC
885 894 903 912 921 930 939

Ser Asn Ser Ala Leu Asn Pro Leu Leu Tyr Ala Phe Leu Gly Ser His Phe Arg Gln Ala Phe
AGC AAC TCC GCG CTG AAC CCG CTG CTC TAC GCC TTC CTG GCG TCG CAC TTC CGA CAG GCC TTC
948 957 966 975 984 993 1002

Arg Arg Val Cys Pro Cys Ala Pro Arg Arg Pro Arg Arg Pro Gly Pro Ser Asp
CGC CGC GTC TGC CCC TGC GCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG
1011 1020 1029 1038 1047 1056 1065

Pro Ala Ala Pro His Ala Glu Leu Leu Arg Leu Gly Ser His Pro Ala Pro Ala Arg Ala Gln
CCC GCA GCC CCA CAC CCG GAG CTG CTC CGC CTG GCG TCC CAC CCG GCC CCC GCG AGG GCG CAG
1074 1083 1092 1101 1110 1119 1128

Lys Pro Gly Ser Ser Gly Leu Ala Ala Arg Gly Leu Cys Val Leu Gly Glu Asp Asn Ala Pro
AAG CCA GCG AGC AGT GCG CTG GCC GCG CGC GCG CTG TGC GTC CTC GCG GAG GAG AAC GCC CCT
1137 1146 1155 1164 1173 1182 1191

Leu TER
CTC TGA

FIG.1C

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CCA CAG TCC CAG GAC GCA ATC TGT GAA GGC TGC CTG GAG GAG GAG GGC GAC AGG GCC
 9 18 27 36 45 54
 ATG GCC GCA GAG GCG ACG TTG GGT CCG AAC GTG AGC TGG TGG GCT CCG TCC AAC GCT TCG GGA TGC
 69 78 87 96 105 114 123
 CCG GGC TGC GGT GTC AAT GCC TCG GAT GGC CCA GGC TCC GCG CCA AGG CCC CTG GAT GCC TGG CTG
 135 144 153 162 171 180 189
 GTG CCC CTG TTT TTC GCT GCC CTA ATG TTG CTG GCG CTA GTC GGG AAC TCA CTG GTC ATC TTC GTT
 201 210 219 228 237 246 255
 ATC TGC CGC CAC AAG CAC ATG CAG ACC GTC ACC AAT TTC TAC ATC GCT AAC CTG GCG GCC ACA GAT
 267 276 285 294 303 312 321
 GTC ACT TTC CTT CTG TGC TGC GTA CCC TTC ACC GCG CTC CTC TAT CCG CTG CCC ACC TGG GTG CTG
 333 342 351 360 369 378 387
 GGA GAC TTC ATG TGC AAA TTC GTC AAC TAC ATC CAG CAG GTC TCG GTG CAA GCC ACA TGT GCC ACT
 399 408 417 426 435 444 453
 TTG ACA GCC ATG AGT GTG GAC GCG TGG TAC GTG ACT GTG TTC CCG CTG CGT GCA CTT CAC CGC CGC
 465 474 483 492 501 510 519
 ACT CCG CGC CTG GCC CTG ACT GTC AGC CTT AGC ATC TGG GTG GGT TCC GCA GCT GTT TCC GCC CCG
 531 540 549 558 567 576 585
 GTG CTG GCT CTG CAC CGC CTG TCG CCC GGG CCT CAC ACC TAC TGC AGT GAG GCG TTT CCC AGC CGT
 597 606 615 624 633 642 651

FIG. 2A

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GCC CTG GAG CGC GCT TTC GCG CTC TAC AAC CTG CTG GCC CTA TAC CTG CTG CCG CTG CTC GCC ACC
663 672 681 690 699 708 717

TGC GCC TGC TAC GGT GCC ATG CTG CCG CAC CTG GCG GCC GCT GTA CCG CCC GCA CCC ACT GAT
729 738 747 756 765 774 783

GCC GCC CTG CAG GGG CAG CTG CTA GCA CAG CCG GCT GGA GCA GTG CCG ACC AAG GTC TCC CGG CTG
795 804 813 822 831 840 849

GTG GCC GCT GTC CTC CTC TTC GCC GCC TGC TGG GGC CCG ATC CAG CTG TTC CTG GTG CTT CAA
861 870 879 888 897 906 915

GCC CTG CCG CTC GGG CCG CTG GCA CCC TCG AAG CTA TGC GCC TAC GCG CTC AAG ATC TGG GCT CAC
927 936 945 954 963 972 981

TGC ATG TCC TAC AGC AAT TCT GCG CTC AAC CCG CTG CTC TAT GCC TTC CTG GGT TCC CAC TTC AGA
993 1002 1011 1020 1029 1038 1047

CAG GCC TTC TGC CCG GTG TGC CCC TGC GCG CCG CAA CCG CAG CGT CCG CCC CAC CCG TCA GCG CAC
1059 1068 1077 1086 1095 1104 1113

TGC GAC CGA GCC GCA CCC CAT AGT GTG CCG CAC AGC CCG GCT CCG CAC CCT GTC CCG GTG AGG ACC
1125 1134 1143 1152 1161 1170 1179

CCC GAG CCT GGG AAC CCT GTG CTC CAC TCG CCC TCT GTT CAG GAT GAA CAC ACT GCC CCA CTC TGA
1191 1200 1209 1218 1227 1236 1245

GCT GCC

FIG.2B

MHTVATSGPN ASWGAPANAS GCPGCGANAS DGPVPSRAV DAWLVPLFFA ALMLLGLVGN 60
SLVIYVICRH KPMRTVTNFY IANLAATDVT FLLCCVPFTA LLYPLPGWVL GDFMCKFVNY 120
IQQVSVQATC ATLTAMSVDR WYVTVFPLRA LHRRTPLRAL AVSLSIWGS AAVSAPVLAL 180
HRLSPGPRAY CSEAFPSRAL ERAFALYNLL ALYLLPLLAT CACYAAMLRH LGRVAVRPAP 240
ADSALGGQVL AERAGAVRAK VSRLVAAVVL LFAACWGPIQ LFLVLQALGP AGSMHPRSYA 300
AYALKTWAHC MSYNSALNP LLYAFLGSHF RQAFRRVCPC APRRRRPRR PGPSDPAAPH 360
AELLRLGSHIP APARAQKPCS SGLAARGLCV LGEDNAPL 398

FIG.3

MAAEATLGPN VSMWAPSNAS GCPGCGVNAS DGPGSAPRPL DAWLVPLFFA ALMLLGLVGN SLVIFVICRH
KHMQTVTNFY IANLAATDVT FLLCCVPFTA LLYPLPTWVL GDFMCKFVNY IQQVSVQATC ATLTAMSVDR
WYVTVFPLRA LHRRTPLRAL TVSLSIWGS AAVSAPVLAL HRLSPGPHTY CSEAFPSRAL ERAFALYNLL
ALYLLPLLAT CACYGAMLRH LGRAAVRPAP TDGALQGQLL AQRAGAVRTK VSRLVAAVVL LFAACWGPIQ
LFLVLQALPL GGLAPSKLCA YALKIWAHGM SYSNSALNPL LYAFLGSHFR QAFCRVCPCG PQRQRPHAS
AHSDDRAAPHS VPHSRAAHPV RVRTPEGPNP VHSPPSVQDE HTAPL

FIG.4

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CCA CAG TCC CAG GAC GCA ATC TGT GAA GGC TGC CTG GAG GAG GAG GGC GAC AGG GCC
 9 18 27 36 45 54

Met Ala Ala Glu Ala Thr Leu Gly Pro Asn Val Ser Trp Trp Ala Pro Ser Asn Ala Ser Gly Cys
 ATG GCC GCA GAG GCG ACG TTG GGT CCG AAC GTG AGC TGG TGG GCT CCG TCC AAC GCT TCG GGA TGC
 69 78 87 96 105 114 123

Pro Gly Cys Gly Val Asn Ala Ser Asp Gly Pro Gly Ser Ala Pro Arg Pro Leu Asp Ala Trp Leu
 CCG GGC TGC GGT GTC AAT GCC TCG GAT GGC CCA GGC TCC GCG CCA AGG CCC CTG GAT GCC TGG CTG
 135 144 153 162 171 180 189

Val Pro Leu Phe Phe Ala Ala Leu Met Leu Leu Gly Leu Val Gly Asn Ser Leu Val Ile Phe Val
 GTG CCC CTG TTT TTC GCT GCC CTA ATG TTG CTG GGG CTA GTC GCG AAC TCA CTG GTC ATC TTC GTT
 201 210 219 228 237 246 255

Ile Cys Arg His Lys His Met Gln Thr Val Thr Asn Phe Tyr Ile Ala Asn Leu Ala Ala Thr Asp
 ATC TGC CGC CAC AAG CAC ATG CAG ACC GTC ACC AAT TTC TAC ATC GCT AAC CTG GCG GCC ACA GAT
 267 276 285 294 303 312 321

Val Thr Phe Leu Leu Cys Cys Val Pro Phe Thr Ala Leu Leu Tyr Pro Leu Pro Thr Trp Val Leu
 GTC ACT TTC CTT CTG TGC TGC GTA CCC TTC ACC GCG CTC CTC TAT CCG CTG CCC ACC TGG GTG CTG
 333 342 351 360 369 378 387

Gly Asp Phe Met Cys Lys Phe Val Asn Tyr Ile Gln Gln Val Ser Val Gln Ala Thr Cys Ala Thr
 GGA GAC TTC ATG TGC AAA TTC GTC AAC TAC ATC CAG CAG GTC TCG GTG CAA GCC ACA TGT GCC ACT
 399 408 417 426 435 444 453

FIG.5A

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Leu Thr Ala Met Ser Val Asp Arg Trp Tyr Val Thr Val Phe Pro Leu Arg Ala Leu His Arg Arg
 TTG ACA GCC ATG AGT GTG GAC CGC TGG TAC GTG ACT GTG TTC CCG CTG CCG TCC GCA CTT CAC CGC CGC
 465 474 483 492 501 510 519

 Thr Pro Arg Leu Ala Leu Thr Val Ser Leu Ser Ile Trp Val Gly Ser Ala Ala Val Ser Ala Pro
 ACT CCG CGC CTG GCC CTG ACT GTG AGC CTT AGC ATC TGG GTG GGT TCC GCA GCT GTT TCC GCC CCG
 531 540 558 567 576 585

 Val Leu Ala Leu His Arg Leu Ser Pro Gly Pro His Thr Tyr Cys Ser Glu Ala Phe Pro Ser Arg
 GTG CTG GCT CTG CAC CGC CTG TCG CCC GGG CCT CAC ACC TAC TGC AGT GAG CCG TTT CCC AGC CGT
 597 606 615 624 633 642 651

 Ala Leu Glu Arg Ala Phe Ala Leu Leu Leu Tyr Leu Leu Pro Leu Leu Ala Thr
 GCC CTG GAG CGC GCT TTC GCG CTC TAC AAC CTG CTG CTC GCC CTA TAC CTG CTG CCG CTG CTC GCC ACC
 663 672 681 690 699 708 717

 Cys Ala Cys Tyr Gly Ala Met Leu Arg His Leu Gly Arg Ala Ala Val Arg Pro Ala Pro Thr Asp
 TGC GCC TGC TAC GGT GCC ATG CTG CGC CAC CTG GGC CGC GCC GCT GTA CGC CCC GCA CCC ACT GAT
 729 738 747 756 765 774 783

 Gly Ala Leu Gln Gly Gln Leu Leu Ala Gln Arg Ala Gly Ala Val Arg Thr Lys Val Ser Arg Leu
 GGC GCC CTG CAG GGG CAG CTG CTA GCA CAG CGC GCT GGA GCA GTG CGC ACC AAG GTC TCC CGC CTG
 795 804 813 822 831 840 849

 Val Ala Ala Val Val Leu Leu Phe Ala Ala Cys Trp Gly Pro Ile Gln Leu Phe Leu Val Leu Gln
 GTG GCC GCT GTC CTC CTC TTC GCC GCC TGC TGG GGC CCG ATC CAG CTG TTC CTG GTG CTT CAA
 861 870 879 888 897 906 915

FIG.5B

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Ala Leu Pro Leu Gly Gly Leu Ala Pro Ser Lys Leu Cys Ala Tyr Ala Leu Lys Ile Trp Ala His
GCC CTG CCG CTC GGG GGC CTG GCA CCC TCG AAG CTA TGC GCC TAC GCG CTC AAG ATC TGG GCT CAC
927 936 945 954 963 972 981

Cys Met Ser Tyr Ser Asn Ser Ala Leu Asn Pro Leu Leu Tyr Ala Phe Leu Gly Ser His Phe Arg
TGC ATG TCC TAC AGC AAT TCT GCG CTC AAC CCG CTG CTC TAT GCC TTC CTG GGT TCC CAC TTC AGA
993 1002 1011 1020 1029 1038 1047

Gln Ala Phe Cys Arg Val Cys Pro Cys Gly Pro Gln Arg Gln Arg Arg Pro His Ala Ser Ala His
CAG GCC TTC TGC CCG GTG TGC CCC TGC GGC CCG CAA CCG CAG CGT CCG CCC CAC GCG TCA GCG CAC
1059 1068 1077 1086 1095 1104 1113

Ser Asp Arg Ala Ala Pro His Ser Val Pro His Ser Arg Ala Ala His Pro Val Arg Val Arg Thr
TCG GAC CGA GCC GCA CCC CAT AGT GTG CCG CAC AGC CCG GCT GCG CAC CCT GTC CCG GTC AGG ACC
1125 1134 1143 1152 1161 1170 1179

Pro Glu Pro Gly Asn Pro Val Val His Ser Pro Ser Val Gln Asp Glu His Thr Ala Pro Leu
CCC GAG CCT GGG AAC CCT GTG GTG CAC TCG CCC TCT GTT CAG GAT GAA CAC ACT GCC CCA CTC TGA
1191 1200 1209 1218 1227 1236 1245

GCT GCC

FIG.5C

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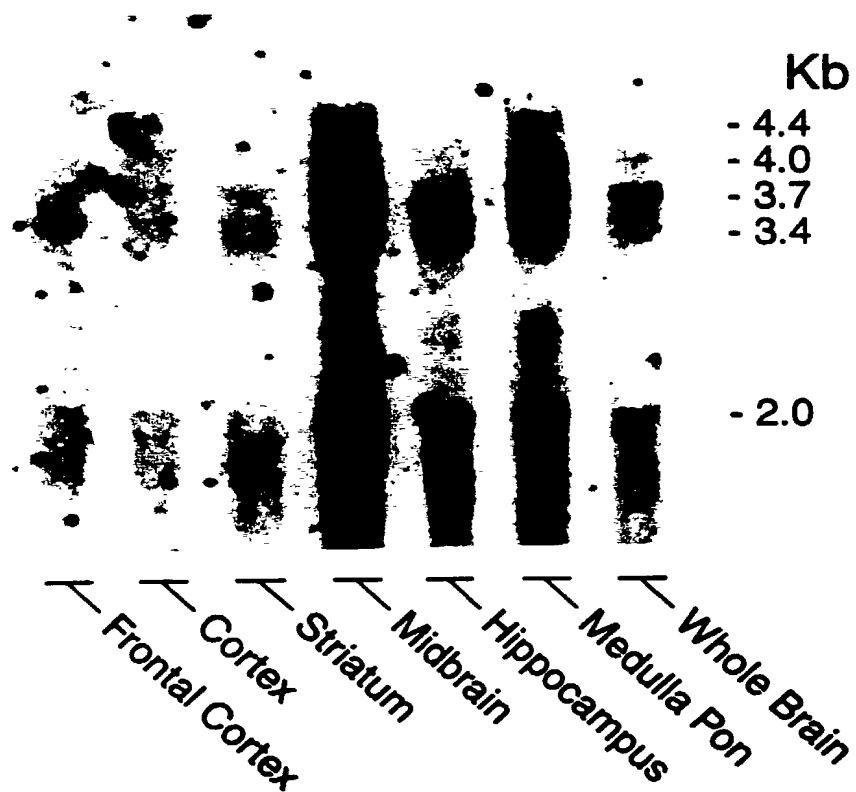


FIG.6

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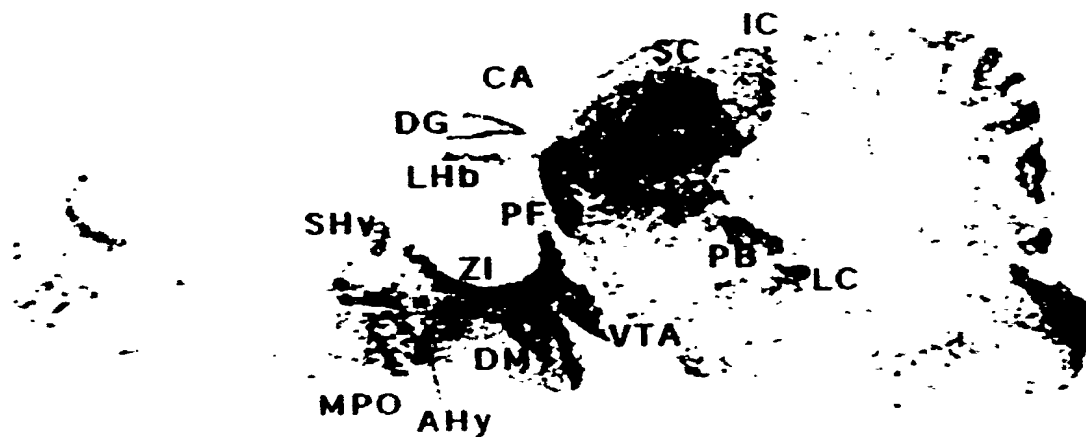


FIG.7A

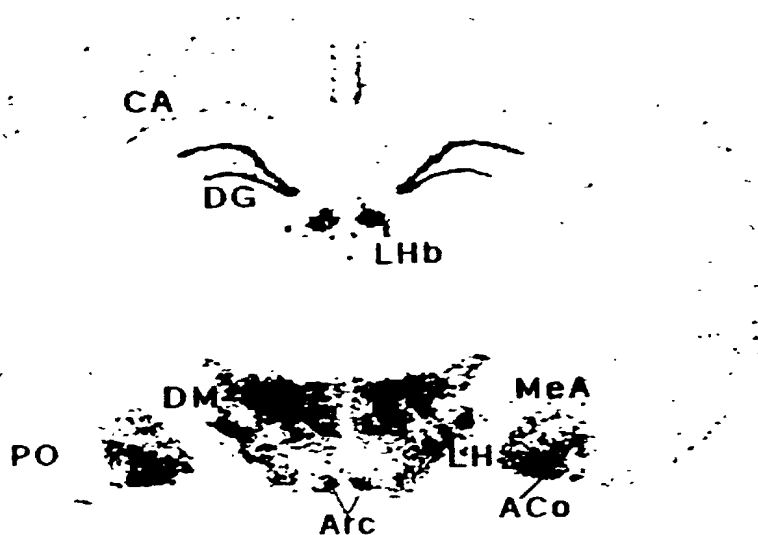


FIG.7B

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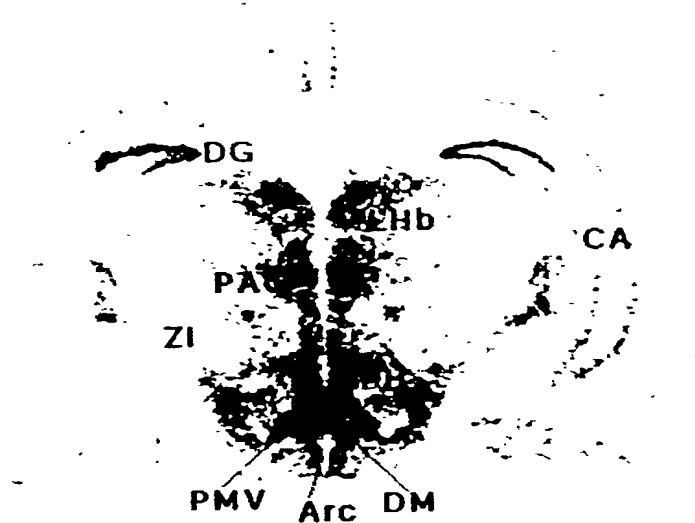


FIG. 7C

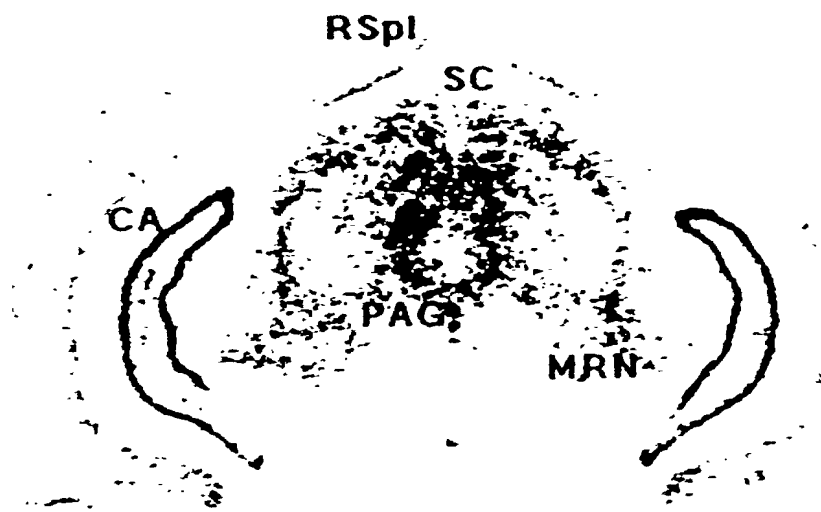


FIG. 7D

09/914,106

33 33
23 23
16 16
8 8
39 39

MAEATLGPVNSWAPNA-SCGNGCGVFAASDGP-
MELAPVNL-SEGNGSDIPEPPAEP
MNGSGSGCAENTISQEG-----
MADIQNIS-----
MEPVPSAPAEIDFSLLANVSDTFPSAFPSASANASGSPG

TRANSMEMBRANE 2

116 116
108 108
99 99
96 96
122 122

GVAFRPLDAMVPLFFAALMLGLVGNLSVIFVJCPHIMQ-----IVTN-FYIANLAAIDVTELLCCVFFIAFLYPLPTWVLGDFMCK
-PLFGIGVENFITLVVFGIFAMGVGNLSVITVMLAPSPGK---PRSTTNLF-ILNLSIADLAAYLLFCDFFGATVVALPTWVLGAEICK
-GSGGQPEAVLPLFFALIFLVGTGVALVLAALLPGGAIV-----STTNLF-ILNLGVADLCFILLCCVFFQATINTLDDWYFGSLCK
-LDSEGSVGAVAVPVTEALIFLLGMVGNLVLAMLLQPGPSANQEPRTTDLF-ILNLAVADLCFILLCCVFFQAAIYITLDAMLFGAIVCK
ARSASSALAIATATAYSAMCAVGLIGNMVMEGINPYTKLK-----IATN-IYIFNLALAD-ALATSTLEDSAKYMETLPTFGEILLCK

TRANSMEMBRANE 4

200 200
194 194
183 183
180 180
209 209

FVNIQQSVQATCATLTAMSVDRMYVTMFLRALHRRIPRALITMSLSIWVGSAAVSAPVLAHLRLSP-GPHITY-CSEAFPSRA--L
FTHYFFTVSMVVSIFTLAAMSVDRYVAIVHSRRSSSLRVSRVALLGVGF-IWALSITAMASPVAYVORLFHRDSNGTLFCMEHPNQL--H
AMHFLIFLTMHASSFTLAAVSLDRYLAIRYPLHSRELRIPIVLAALICQ-IMGLALLFSQPYISYRQSL-ANLIT-VCHPAWSAP---R
TMHLLIYLTMHASSFTLAAVSLDRYLAIRVHQLRSRALRIPVNAARAACQL-VMLLAALFSAPYLSYGTTRY-GALEL-CVPAWEDA---R
AMLSIDYNNMFTSIFTLTMVSDRYIAVCHPVKALDFRTPAKAKLINIC-LWVLSGSGVPIVMVNAVTPRDGA--VVCITLQFPSPSWYV

TRANSMEMBRANE 6

288 288
268 268
259 259
258 258
284 284

ERAFALYNLLALYLLPLATCACYGAWLRHLGRAVPPAPTIDGALLAQ--RAGAVETKVSPLMAVVLFAACWCPJQLFLMLQAL
KKAYVVCFTVFGYLLPLLICFCYAKVNLHKKLK-----NMSKKSEASKKIT--AQTMVVMVVFISMLPHHV-IHLWA-
RRAMDCTFVSYPPLVLSLTIVARTLRVLR-TV--DFV-----TAGSGSQRKRKVT---PMIITJVAVEFCLCWPMPHA-IILCV-
RPRLDVATFAAGYLLPVAVVSLAYQRTILCFILWA-AV--DPA-GA---AAAEARPRATGEA--CPAMLAVALYALCWGPHHA-IILCF-
DVTVKICVFFAFVVPPIIITVCYCLMLRLRS--VP-----LLSGSKEKDRSLRRTITPMQLVNYGAEVVCWAPJHIFVIVW--

FIG.8A

TRANSMEMBRANE 7

GPR54	PLGGLAPSKLCAYALKIMAHQMSYSNSALNPILLVAFLGSHFRQAFCRVCCGPQRORPPHASAHSDPAA-PHSVPHSRAAHFVMPRTPEP	377
Gα IR1	EFIGAFPLTPAS-FFFRITIAHQLAYSNSSVNPITTYAFISENFRKAYKQVFKRVCSNEPHGDAKEKNRIDTPTSTNCTHV	346
Gα IR2	WFGRTPLTRA-TYALRIJLSHLVSIANSNVNPITVYALVSKHFKGFRKIQAGLLRPAPRRASGRVSI LAPGNHSGSMLEQESTDLTQVSEA	348
Gα IR3	WYGRFAFSPA-TYACRLASHGLAYANSCLNPINWLSASRIHFRARFRRLWPCGGRQRIRHHRAHRALEPRVCPASSGYPGDAFPRGWSM	347
DDR	TLVDINRRDPLVVAALHLCIALGYANSSLNRPVLYAFIDENFKRCFRQLCRAPCGGCEPGSLRRPRQATAREVTACTPSDGPJGGAAA	372
GPR54	GNPVMHSPSVQDEHTAPL	395
Gα IR2	ACPLVPPPALPNC TASSRTLDPAC	372
Gα IR3	EPRGDALRGGGETRLTSPRGPO	370

FIG.8B

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		10	20	30	40	50	60
rat	MAAEATLGPNVSWWAPSNASGCPGCGVNASDGPGSAPRPLDAWL	VPLFF	AALML	LGLVGN			
	:	:	:	:	:	:	:
human	MHTVATSGPNASWGAPANASGCPGCGANASDGPVPSRAVD	AWL	VPLFF	AALML	LGLVGN		
		10	20	30	40	50	60
		70	80	90	100	110	120
rat	SLVIFVICRHKHMQTVTNFYIANLAATDVTFLLCCVPFTALLY	YPLPTW	VLGDF	MCKFVNY			
	:	:	:	:	:	:	:
human	SLVIYVICRHKPMRTVTNFYIANLAATDVTFLLCCVPFTALLY	YPLPGW	VLGDF	MCKFVNY			
		70	80	90	100	110	120
		130	140	150	160	170	180
rat	IQQVSVQATCATLTAMSVDRWYVTVFPLRALHRRTPRLAL	TVSL	SIWVGS	AAVSAPVLAL			
	:	:	:	:	:	:	:
human	IQQVSVQATCATLTAMSVDRWYVTVFPLRALHRRTPRLAL	AVSL	SIWVGS	AAVSAPVLAL			
		130	140	150	160	170	180
		190	200	210	220	230	240
rat	HRLSPGPHTYCSEAFPSRALERAFALYNLLALYLLPLL	ATCACY	GAMLRHL	GRAAVRPAP			
	:	:	:	:	:	:	:
human	HRLSPGPRAYCSEAFPSRALERAFALYNLLALYLLPLL	ATCACY	AAMLRHL	GRVAVRPAP			
		190	200	210	220	230	240
		250	260	270	280	290	
rat	TDGALQGQLLAQRAGAVRTKVSRLVAAVVLLFAACWGPI	QLFLVL	QAL-PLGGL	APSKLC			
	:	:	:	:	:	:	:
human	ADSALQGQVLAERAGAVRAKVSRLVAAVVLLFAACWGPI	QLFLVL	QALGPAGS	WHPRSYA			
		250	260	270	280	290	
		300	310	320	330	340	350
rat	AYALKIWAHCMSYSNSALNPLL	YAF	LGSHFRQAF	CRVCPCGPQRQRR	PHASA	HSRAAPH	
	:	:	:	:	:	:	:
human	AYALKTWAHCMSYSNSALNPLL	YAF	LGSHFRQAF	RRVCPCAPRRPRR	PRRPGSPD	PAAPH	
		310	320	330	340	350	360
		360	370	380	390		
rat	SVPHSRAAHPVRVRTPEPGNP	—VHSPSVQDEHT	APL				
	:	:	:	:	:	:	:
human	AELLRLGSHPAPARAQKPGSSGLAARGLCVLGED	NAPL					
		370	380	390			

FIG.9

Please type a plus sign (+) inside this box

SUBSTITUTE for PTO/SB/01 (12-97), DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

**DECLARATION AND
POWER OF ATTORNEY
FOR UTILITY OR DESIGN
PATENT APPLICATION
(37 CFR 1.63)**

☐ Declaration Submitted with Initial Filing **OR** ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e)) required)

Attorney Docket Number 20397P

First Named Inventor Howard, et al.

COMPLETE IF KNOWN

Application Number 09/914,106

Filing Date August 23, 2001

Group Art Unit

Examiner Name

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

G PROTEIN-COUPLED RECEPTOR RESEMBLING-GALANIN RECEPTORS

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 08/23/2001 as United States Application Number or PCT International

Application Number and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed?	
				YES	NO
PCT/US00/04416	PCT	02/22/2000	20397PCT	<input checked="" type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/121,651	02/24/1999	20397PV

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U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/US00/04416	02/22/2000	
60/121,651	02/24/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

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OR

☒ Registered practitioner(s) name/registration number listed below

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Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

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Name	Joseph A. Coppola				
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Address	P.O. Box 2000, RY60-30				
City	Rahway	State	NJ	ZIP	07065-0907
Country	USA	Telephone	(732)594-6734	Fax	(732)594-4720

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])		Family Name or Surname			
ANDREW D.		HOWARD			
Inventor's Signature				Date	
Residence: City	Park Ridge	State	NJ	Country	US
				Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000				
City	Rahway	State	NJ	ZIP	07065-0907

☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
GARY P.				O'NEILL			
Inventor's Signature						Date	
Residence: City	Quebec	State		Country	CA	Citizenship	CA
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
BRIAN				O'DOWD			
Inventor's Signature						Date	
Residence: City	Toronto, Ontario	State		Country	CA	Citizenship	CA
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
SUSAN				GEORGE			
Inventor's Signature	<i>Susan R. George</i>					Date	NOV 9/01
Residence: City	Toronto, Ontario	State	CA	Country	CA	Citizenship	CA
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		

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Substitute for PTO/SB/01 (12-97), Declaration for Utility or Design Patent Application

Approved for use through 9/30/2000 OMB 651-0032

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POWER OF ATTORNEY
FOR UTILITY OR DESIGN
PATENT APPLICATION
(37 CFR 1.63)**

☐ Declaration
Submitted
with Initial
Filing

OR

☒ Declaration
Submitted after Initial
Filing (surcharge
(37 CFR 1.16 (e))
required)

Attorney Docket Number

20397P

First Named Inventor

Howard, et al.

COMPLETE IF KNOWN

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09/914,106

Filing Date

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Group Art Unit

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G PROTEIN-COUPLED RECEPTOR RESEMBLING-GALANIN RECEPTORS

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 08/23/2001 as United States Application Number or PCT International

Application Number and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

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				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>

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60/121,651	02/24/1999	

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☒ Registered practitioner(s) name/registration number listed below

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Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

Direct all correspondence to: ☒ Customer Number or Bar Code Label

000210

Name	Joseph A. Coppola				
Address	Merck & Co, Inc. - Patent Department				
Address	P.O. Box 2000, RY60-30				
City	Rahway	State	NJ	ZIP	07065-0907
Country	USA	Telephone	(732)594-6734	Fax	(732)594-4720

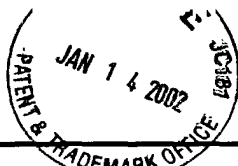
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])		Family Name or Surname					
ANDREW D		HOWARD					
Inventor's Signature		Date					
Residence: City	Park Ridge	State	NJ	Country	US	Citizenship	US
Post Office Address	Merck & Co, Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		

☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

Please type a plus sign (+) inside this box



Approved for use through 9/30/2000 OMB 651-0032
SUBSTITUTE for PTO/SB/02A (3-97), Declaration (Additional Inventors)

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S) Supplemental Sheet

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
GARY P.				O'NEILL			
Inventor's Signature						Date	
Residence: City	Quebec	State		Country	CA	Citizenship	CA
Post Office Address	Merck & Co., Inc., P O Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
BRIAN				O'DOWD			
Inventor's Signature						Date	
Residence: City	Toronto, Ontario	State		Country	CA	Citizenship	CA
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
SUSAN				GEORGE			
Inventor's Signature						Date	
Residence: City	Toronto, Ontario	State		Country	CA	Citizenship	CA
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
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Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		

**DECLARATION AND
POWER OF ATTORNEY
FOR UTILITY OR DESIGN
PATENT APPLICATION
(37 CFR 1.63)**☐ Declaration
Submitted
with Initial
Filing

OR

☒ Declaration
Submitted after Initial
Filing (surcharge
(37 CFR 1.16 (e))
required)

Attorney Docket Number 20397P

First Named Inventor Howard, et al.

COMPLETE IF KNOWN

Application Number 09/914,106

Filing Date August 23, 2001

Group Art Unit

Examiner Name

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

G PROTEIN-COUPLED RECEPTOR RESEMBLING-GALANIN RECEPTORS

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 08/23/2001 as United States Application Number or PCT International

Application Number and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed?	
				YES	NO
PCT/US00/04416	PCT	02/22/2000	20397PCT	<input checked="" type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/121,651	02/24/1999	20397PV

Please type a plus sign (+) inside this box

Substitute for PTO/SB/02B-2-99 Declaration for Utility or Design Patent Application

DECLARATION AND POWER OF ATTORNEY for Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information known to me to be material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/US00/04416	02/22/2000	
60/121,651	02/24/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith

☐ Customer Number OR
☒ Registered practitioner(s) name/registration number listed below

Place Customer Number
Bar Code Label here

Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

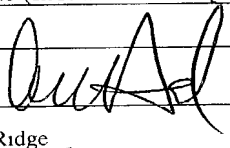
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Name	Joseph A. Coppola				
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Address	P.O. Box 2000, RY60-30				
City	Rahway	State	NJ	ZIP	07065-0907
Country	USA	Telephone	(732)594-6734	Fax	(732)594-4720

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])		Family Name or Surname	
ANDREW D.		HOWARD	
Inventor's Signature		Date	11/13/01
Residence: City	Park Ridge	State	NJ
		Country	US
		Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000		
City	Rahway	State	NJ
		ZIP	07065-0907

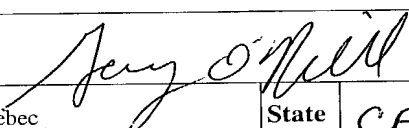
☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

Please type a plus sign (+) inside this box

Approved for use through 9/30/2000 OMB 651-0032
SUBSTITUTE for PTO/SB/02A (3-97), Declaration (Additional Inventors)

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S) Supplemental Sheet

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])				Family Name or Surname				
GARY P.				O'NEILL				
Inventor's Signature					Date		12-Nov-01	
Residence: City		Quebec	State	CAN	Country	CA	Citizenship	CA
Post Office Address		Merck & Co., Inc., P.O. Box 2000						
City		Rahway	State	NJ	ZIP	07065-0907		
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Given Name (first and middle [if any])				Family Name or Surname				
BRIAN				O'DOWD				
Inventor's Signature					Date			
Residence: City		Toronto, Ontario	State		Country	CA	Citizenship	CA
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City		Rahway	State	NJ	ZIP	07065-0907		
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Inventor's Signature					Date			
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Inventor's Signature					Date			
Residence: City			State		Country		Citizenship	
Post Office Address		Merck & Co., Inc., P.O. Box 2000						
City		Rahway	State	NJ	ZIP	07065-0907		

Please type a plus sign (+) inside this box

Substituted for PTO/SB/01 (12/97), Declaration for Utility or Design Patent Application

Approved for use through 9/30/2000 OMB 651-0032

**DECLARATION AND
POWER OF ATTORNEY
FOR UTILITY OR DESIGN
PATENT APPLICATION
(37 CFR 1.63)**



Declaration
Submitted
with Initial
Filing

OR



Declaration
Submitted after Initial
Filing (surcharge
(37 CFR 1.16 (e))
required)

Attorney Docket Number

20397P

First Named Inventor

Howard, et al.

COMPLETE IF KNOWN

Application Number

09/914,106

Filing Date

August 23, 2001

Group Art Unit

Examiner Name

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the specification of which



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As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

☐ Customer Number OR
☒ Registered practitioner(s) name/registration number listed below

Place Customer Number
Bar Code Label here

Name	Registration Number	Name	Registration Number
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City	Rahway	State	NJ	ZIP	07065-0907
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Name of Sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])		Family Name or Surname			
ANDREW D.		HOWARD			
Inventor's Signature				Date	
Residence: City	Park Ridge	State	NJ	Country	US
				Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000				
City	Rahway	State	NJ	ZIP	07065-0907

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DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
GARY P.				ONEILL			
Inventor's Signature						Date	
Residence: City	Quebec	State		Country	CA	Citizenship	CA
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
BRIAN				O'DOWD			
Inventor's Signature	<i>Brian O'Dowd</i>					Date	7/11/01
Residence: City	Toronto, Ontario	State	CAK	Country	CA	Citizenship	CA
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
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Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
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SUSAN				GEORGE			
Inventor's Signature						Date	
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